Original Article

موضوع أصيل

THE POTENTIAL PREVENTIVE ROLE OF VITAMIN C AGAINST STRUCTURAL CHANGES IN MALE RATS' LIVER INDUCED BY TRAMADOL

الدور الوقائي المحتمل للفيتامين C ضد التغيرات البنيوية الكبدية الناجمة عن المعالجة باستخدام Tramadol عند ذكور الفئران

Rana M. Ahmad M.B.Ch.B., MSc; Abdul-Jabbar Y. Al-Hubaity, M.B.Ch.B., Ph.D
Nadwa S. Alazow, M.B.Ch.B., F.I.B.MS.
د. رنا مصطفى أحمد، د. عبد الجبار ياسين الحبيطي، د. ندوى صبحي العزو

ملخص البحث

هدف البحث: تهدف هذه الدراسة إلى استقصاء التأثيرات السامة لعقار Tramadol على نسيج الكبد عند ذكور الجرذان البيضاء البالغة والدور الوقائي المحتمل لإعطاء الفيتامين C.

طرق البحث: شملت الدراسة 30 من ذكور الجرذان البيضاء البالغة، تم تقسيم الحيوانات إلى ثلاث مجموعات متساوية. المجموعة الأولى هي مجموعة الشاهد، تم حقن حيوانات هذه المجموعة بالماء المقطر يومياً. المجموعة الثانية وقد تم حقنها بـ Tramadol بجرعة 50 ملغ/كغ من وزن الجسم يومياً. المجموعة الثالثة وتم حقنها بالفيتامين C بجرعة 100 ملغ/كغ من وزن الجسم قبل نصف ساعة من حقن Tramadol بنفس الجرعة المستخدمة في المجموعة الثانية. تم الحقن لدى جميع الحيوانات في غشاء البريتوان ولمدة أربعة أسابيع. تم في نهاية التجربة قتل جميع الحيوانات بعد تخديرها بمادة الإيثر، ثم تم أخذ الكبد ووضعه في مثبت ومعالجته للحصول على قوالب البارافين وإعداده للفحص المجهري.

النتائج: لوحظ حدوث تغيراتٍ نسيجية لدى الجرذان المعالجة بـ Tramadol مقارنةً بمجموعة الشاهد، وتمثلت هذه التغيرات بتوسع واحتقان في الوريد المركزي، الوريد البابي وأشباه الجيوب الكبدية، زيادة ارتشاح الخلايا الالتهابية وحيدة النواة مع فرط تنسج ملحوظ في خلايا كوبفر. بالاضافة إلى ذلك، ظهور فجوات في خلايا الكبد مع فرط تنسج في الأقنية الصفراوية مع ترسب أليافٍ الكولاجين في النسيج الكبدي وخصوصاً حول المنطقة البابية. لوحظ تحسن في جميع هذه التغيرات البنيوية في الكبد لدى إعطاء الفيتامين C قبل إعطاء Tramadol.

الاستنتاجات: يسبب Tramadol تغيراتٍ بنيوية في نسيج الكبد، إلا أن إضافة الفيتامين C يؤدي إلى التخفيف من هذه التغيرات.

ABSTRACT

Objective: *The aim of the study is to detect the toxic effects of tramadol on the histological structure of the liver tissue in adult male albino rats and the possible protective role of vitamin C.*

Methods: *Thirty adult albino rats were used in this study. The animals were divided into three equal groups: Group A (Control group): In this group animals were injected with distilled water. Group B (Tramadol treated group, TGI): Animals were given tramadol in a dose of 50 mg/kg daily. Group C (Group treated with tramadol plus vitamin C, TGII): Animals were given vitamin C 100 mg/Kg half hour prior to tramadol injection in the same dose as in TGI. All animals were injected intraperitoneally (I.P) for four weeks. At the end of*

*Rana M. Ahmad M.B.Ch.B., MSc, Assistant Lecturer, Department of Anatomy, College of Medicine, University of Mosul, Mosul, Iraq.
E-mail: dr.rana.mustafa.76@gmail.com.
*Abdul-Jabbar Y. Al-Hubaity, M.B.Ch.B., Ph.D, Professor, College of Medicine, University of Mosul, Mosul, Iraq.
*Nadwa S. Alazow, M.B.Ch.B., F.I.B.MS, Assistant Professor, College of Medicine, University of Mosul, Mosul, Iraq.

the experiment, the rats were killed after anaesthetized by ether then the liver was removed and preserved in a fixative and processed to get paraffin wax block and prepared for microscopic examination.

Results: *Histological changes has been found in the tramadol treated group TGI compared to the control group including dilatation and congestion of central vein, portal vein and sinusoids. Also increased infiltration of mononuclear inflammatory cells (MNCs) with prominent kupffer cells hyperplasia. In addition, the hepatocytes showed vacoulation with bile duct hyperplasia and increased deposition of collagen fibers in liver parenchyma mainly around portal area. All these structural changes in liver were ameliorated by giving vitamin C prior to tramadol.*

Conclusions: *Tramadol caused structural changes in liver tissues and the addition of vitamin C ameliorates these changes.*

INTRODUCTION

Tramadol is a centrally acting opioid analgesic which is mainly used for treatment of moderate to severe pain.[1] The mechanism of its analgesic action is complex. Most reports suggested that the analgesic activity occur as result of opioid and non-opioid mechanisms. It also inhibits the neuronal reuptake of norepinephrine and serotonin, as do the antidepressant drugs such as amitriptyline.[2] Tramadol is rapidly and almost completely absorbed after oral administration. The mean peak plasma concentration occurs after 2 hours and its bioavailability is approximately 70% as a result of the first-pass metabolism in the liver and the mean half-life is 6 hours. The complete absorption of tramadol occurs in the upper portion of small.[3] Tramadol is metabolized in the liver by two principal pathways into M1 and M2, by only M1 is pharmacologically active. Its selectivity for μ-receptors has been demonstrated, showing higher affinity for opioid receptors than the parent drug.[4] So, it is transformed in the liver to M1, which itself is an active substance and 2-4 times more effective and potent than tramadol.[5] Approximately 30% of the dose is excreted in the urine as unchanged drug, while 60% of the dose is excreted as metabolites.[6] Repeated tramadol administration might lead to the accumulation of toxic metabolites in the body, increase the risk for its toxico-kinetics effects and/or decrease the clearance of tramadol, thus increasing its toxicity.[7]

Vitamin C, a water-soluble vitamin is as potent micronutrient that has two main functions as an antioxidant and as an enzyme cofactor.[8] It performances as a biological antioxidant that scavenges free radicals and other reactive nitrogen and oxygen species by donating an electron to free radical species there by interrupting the radical chain reaction.[9] So, even in small amounts; it can protect indispensable molecules in the body, as proteins, lipids and nucleic acids against oxidative harm.[10]

METHODS

The current study was done in animal house in veterinary college/ university of Mosul as a part of Ph.D thesis from April 2018 to January 2019. Thirty adult male Wister albino rats weighing about 220-250 mg, and aged three months were used in this study and kept at controlled room temperature (23-25 Cº) with a 12 hours light/dark cycle, and placed in plastic cages using homogenized wood shaving as bedding for acclimatization one week before the experiment. Tramadol and vitamin C ampoules were used in the current study. The animals were divided into three equal groups as following:

Group A (control group): In this group animals were injected with distilled water. Group B (treated with tramadol) (TGI): Animals were given tramadol in a dose of 50 mg/kg daily. Group C (treated with tramadol plus vitamin C) (TGII): Animals were given vitamin C 100 mg/kg half hour prior to tramadol injection in the same dose as in TGI. All animals were injected intraperitoneally (I.P) for four weeks. At the end of the experimental period, the rats were anaesthetized by ether then killed and the liver was rapidly dissected out carefully then the specimens were perfused with saline followed by fixation with 10% neutral buffered formalin, and processed for light microscopic study to get paraffin sections of 5 μm thickness. Sections were stained with Haematoxylin and Eosin (H&E) and Masson's Trichrome stains.

RESULTS

- Group A (Control group): The liver sections looks with normal central veins, into which drains a converging series of sinusoids with interconnecting plates of hepatocytes that surrounds each sinusoidal channel and run between the central terminal hepatic venules and the periphery of the lobule, Figure 1. Peripherally arranged portal triads is seen and, each contain terminal branches of hepatic artery, portal vein and small tributary of the bile duct surrounded by normal amount of collagen fibers, Figure 2.

- Group B (treated with tramadol) (TGI): Sections of liver taken from this group showed disturbance changes in liver architecture characterized by dilatation and congestion of central vein, portal vein in portal area and sinusoids, Figure 3. Increased infiltration of mononuclear inflammatory cells (MNCs) within the hepatocytes and sinusoids with prominent Kupffer cells hyperplasia, Figure 4. The hepatocytes showed vacuolation, Figure 5. While bile duct showed prominent hyperplasia Figure 6, and increased deposition of collagen fibers in liver parenchyma mainly around portal area, Figure 7.

- Group C (treated with tramadol plus vitamin C) (TG II): Sections of this group showed amelioration in most of the changes which has been seen in TGI, with more preserved liver architecture as mild dilatation and little congestions of blood vessels, with less degree of hyperplasia in Kupffer cells (Figure 8) with few vacuolation in the hepatocytes, Figure 9. Less MNCs infiltration within sinusoids and around blood vessels, with less degree of bile duct hyperplasia, Figure 10. In addition to mild deposition of collagen fibers around portal area, Figure 11.

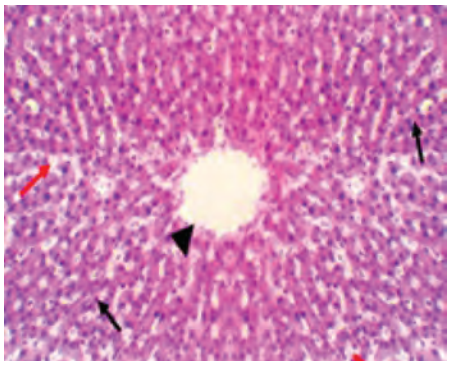

Figure 1. Photomicrogragh of rat's liver of control goup showing normal tissue, central vein (arrow head) hepatocytic plates (black attows), sinusoids (red arrows). (H&EX100).

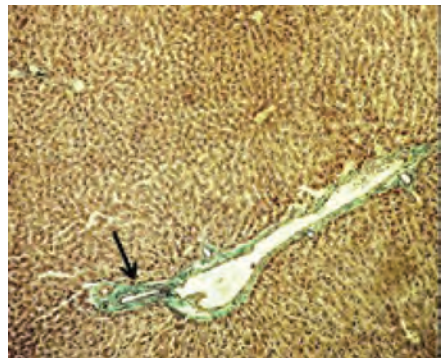

Figure 2. Photomicrograph of rats liver of control group showing normal amount of collagen fibers around the portal area (black arrow)(Masson's trichrome stainX100).

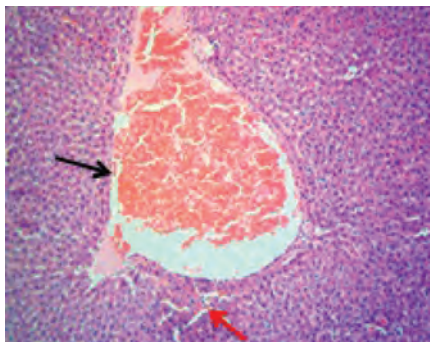

Figure 3. Photomicrogragh of rat's liver of TG I showing congestion and dilatation vein (black arrow) and sinusoids (red arrow) (H&EX100).

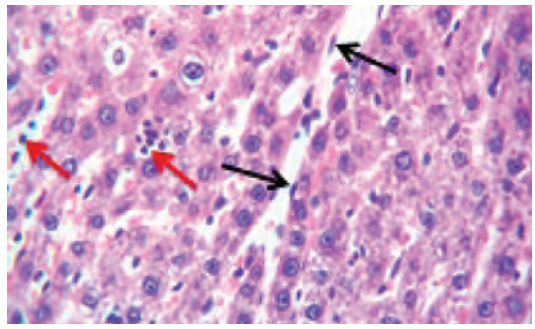

Figure 4. Photomicrogragh of rat's liver of TG I showing kupffer cells hyperplasia (black arrows) and MNCs infiltration within sinusoids (red arrows) (H&EX400).

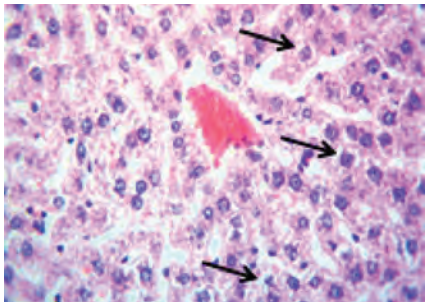

Figure 5. Photomicrogragh of rat's liver of TG I showing diffuse vacoulation (black arrow) (H&EX400).

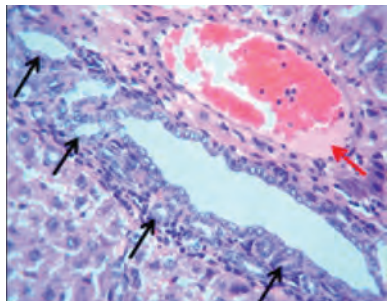

Figure 6. Photomicrogragh of rat's liver of TG I showing bile duct hyperplasia (black arrows) with congested dilated portal vein (red arrow) (H&EX400).

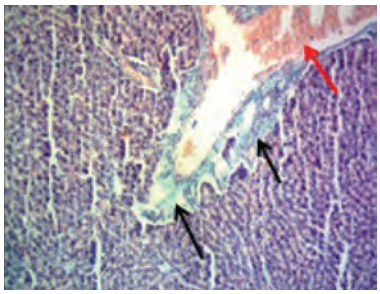

Figure 7. Photomicrogragh of rat's liver of TG I showing increased collagen fibers (black arrows) around congested dilated portal vein (red arrows) (Masson's trichrome stain X100).

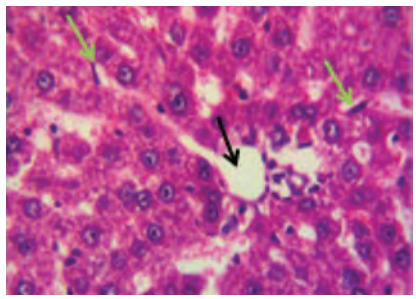

Figure 8. Photomicrogragh of rat's liver of TG II showing nearly normal central vein (black arrow) mild kupffer cell hyperplasia (green arrow) (H&EX400).

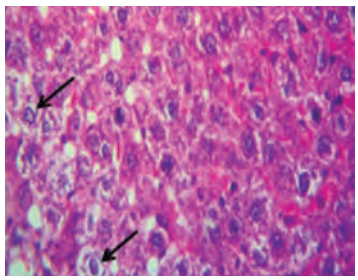

Figure 9. Photomicrogragh of rat's liver of TG II showing mild degree of vacoulation within the hepatocytes (black arrow) (H&EX400).

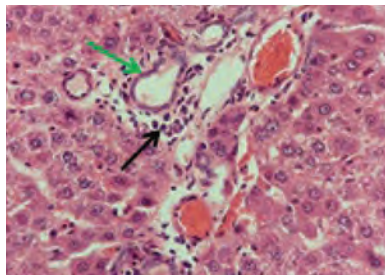

Figure 10. Photomicrogragh of rat's liver of TG II showing less MNCs infiltration (black arrow). less bile ducts hyperplasia (green arrow) (H&EX400).

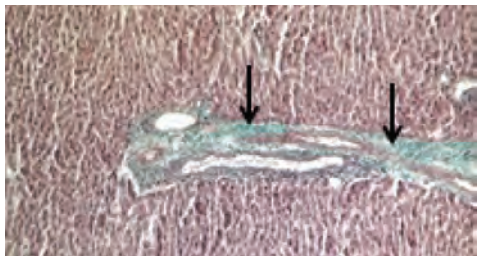

Figure 11. Photomicrogragh of rat's liver of TG II showing less deposition of collagen fibers around portal area (black arrow) (Masson;s trichrome stain X100).

DISCUSSION

Tramadol is metabolized in the liver and so may have hepatotoxic adverse effects on animal body during its metabolism.[11]

Group B (treated with tramadol) (TGI): In comparison to the control group, the histopathological finding of TGI showed changes including the disturbance of liver architecture, dilatation and congestion of central vein, sinusoids and portal vein were evident in this group. Such finding was reported by other workers,[12-14] and explanation for such histological changes could be attributed to the harmful effect of tramadol on heart. It is well known that the mammalian heart is affected by opioids administration.[15] Other clear finding of the current study is increased (MNCs) infiltration among hepatocytes, and sinusoids and particularly around the blood vessels in portal area and such finding was reported by others,[16,17] while others found there is aggregation of lymphocytes only around central vein when given tramadol orally to mice in dose 3 mg/kg for only two weeks,[18] and this might be due to short duration of treatment or small dose. Such abundance of inflammatory cells is a prominent response of body tissues facing any harmful impacts.[19]

Other obvious change was cytoplasmic vacuolation, which reported by other studies,[12,14,20] such vacoulation has been regarded to be an alteration produced to collect the injurious substances in the cells.[12] Another change was clear bile ducts hyperplasia which was reported by other worker,[16,17] while Rabei HM,[21] has not reported bile duct hyperplasia after oral administration of rats with tramadol for 15 days and this could be due to short period of treatment.

Moreover, increased collagen fibers deposition in hepatic tissues and mainly around portal area is detected by using Masson's trichrome in the current study which is similar to that as noticed by others,[13,17] but against studies were done by Rabei HM,[21] and Hafez et al,[22] and this bile ducts hyperplasia with periportal fibrosis may be result as a consequence of a toxic insult.[23]

Increased number of Kupffer cells was evident in this group which was reported by other workers,[12, 20] while others describe there is only elongated of Kupffer cells after treatment rats with tramadol.[18] The reason for Kupffer cells hyperplasia might be correlated with the amount of injury to the hepatic tissue induced by tramadol intoxication, and represents a defense mechanism of detoxification and might be contributed to hepatic oxidative stress.[24]

In other word, treatment with tramadol for long period has adverse effect on liver tissue, and such toxicity can occurred via oxidative stress.[13] The oxidative stress is result from an imbalance between the excessive formation of ROS and inadequate antioxidant defenses, the ROS has wide-ranging effects, but three reactions are mainly relevant to cell injuries which are lipid peroxidation of membranes, oxidative alteration of proteins and lesions in DNA.[25]

Group C (treated with tramadol and vitamin C) (TG II): In the current study, concomitant use of vitamin C along with tramadol produced improvement in histopathological findings in this group in compared with tramadol treated group only (TG I). There is no similar study concerning hepatoprotective effect of vitamin C against tramadol, but there are other researchers studying the hepatoprotective role of vitamin C against other agent as cisplatineum was demonstrated by Ahmed RM[26] after she injected rats I.P. with same dose of vitamin C (i.e.100 mg/kg of vitamin C) prior to cisplatineum. Also Abdul-Qader and his colleague[27] proved the hepato-protective effect of vitamin C against formaldehyde. The mechanism by which vitamin C reduces the side effects of tramadol is mostly due to its acts as an antioxidant, that scavenges reactive oxygen species and through which, the oxidative stress and its related complications are reduced.[28]

CONCLUSIONS

Although tramadol plays an important role in pain management, but its toxic effects should be kept in mind, as the current study proves tramadol cause toxic effect in hepatic tissues of male albino rats. But such toxicity can be ameliorated by use of vitamin C as supplement during tramadol treatment.

REFERENCES

1. *Nossaman VE, Ramadhyani U, Kadowitz PJ, et al. Advances in perioperative pain management: Use of medications with dual analgesic mechanisms, tramadol and tapentadol. Anesthesiol Clin 2010;28(4):647-66.*
2. *Gillman PK. Monoamine oxidase inhibitors, opioid analgesics and serotonin toxicity. Br J Anaesth 2005;95(4):434-41.*
3. *Saleem R, Iqbal R, Abbas MN, et al. Effects of tramadol on histopathological and biochemical parameters in mice (Mus musculus). G J Pharmacol 2014;8(1):14-9.*
4. *Halling J, Weihe P, Brosen K. CYP2D6 polymorphism in relation to tramadol metabolism: A study of faroese patients. Ther Drug Monit 2008;30(3):271-5.*
5. *Raffa RB. Basic pharmacology relevant to drug abuse assessment: tramadol as example. J Clin Pharm Ther 2008;33(2):101-8.*
6. *Eassa BI, El-Shazly MA. Safety and efficacy of tramadol hydrochloride on treatment of premature ejaculation. Asian J Androl 2013;15(1):138-42.*
7. *Shadnia S, Soltaninejad K, Heydari K, et al. Tramadol intoxication: a review of 114 cases. Hum Exp Toxicol 2008;27(3):201-5.*
8. *Combs GF. The vitamins (4th ed.). Elsevier Science, Burlington. part II. 2012. p. 233-53.*
9. *Kesinger N, Stevens J. Covalent interaction of ascorbic acid with natural products. Phytochem 2009;70(17/18):1930-9.*
10. *Padayatty SJ, Sun AY, Chen Q, et al. Vitamin C: intravenous use by complementary and alternative medicine practitioners and adverse effects. PloS One 2010;5(7):e11414.*
11. *Wu WN, Mcknown LA, Gauthier AD, et al. Metabolism of analgesic tramadol hydrochloride in rat and dog. Xenobiotica 2001;31(7):423-41.*
12. *Alhamedi A. Histopathological and histochemical study of tramadol on rabbit's liver and kidney. MSc. Thesis. Faculty of science, Islamic University of Gaza. 2015;16-23.*
13. *Awadalla EA, Salah-Eldin AE. Histopathological and molecular studies on Tramadol mediated hepatorenal toxicity in rats. J Pharm Biol Sci 2015;10(6):90-102.*
14. *Ali O Kh, Al-Jmor SA, Al-Graibawi MA. Effects of tramadol on histopathological and biochemical parameters in male rabbits. Am J Life Sci 2015;3(3):85-90.*
15. *Gupta T, Prakash P, Aronow WS. Association between opioid abuse/dependence and outcomes in hospitalized heart failure patients. Am J Therap 2015;1-7.*
16. *Samaka RM, Girgis NF, Shams TM. Acute toxicity and dependence of tramadol in albino rats: relationship of nestin and notch 1 as stem cell markers. J Am Sci 2012;8(6):313-27.*
17. *Elkhateeb A, El-Khishin I, Megahed O, et al. Effect of Nigella sativa linn oil on tramadol-induced hepato- and nephrotoxicity in adult male albino rats. Toxicol Rep 2015;2:512-9.*
18. *Albarakai AY, Alsbery AE. Evaluation of the hepatoprotective efficacy of Moringa oleifera on tramal-induced liver toxicity in animal modules. Res J Pharm Biol Chem Sci 2016;7(5):1494-501.*
19. *Nwaopara AQ, Odike MAC, Inegbenebor U, et al. The combined effects of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the liver. Pak J Nut 2007;6(6):524-7.*
20. *Badawi SM, Hammad SA, Amin SA, et al. Biochemical, histo-pathological, and immuno-histochemical changes on the liver of adult albino rats due to dependence on tramadol, diazepam, and their combination. Menoufia Med J 2016;29(4):1122-9.*
21. *Rabei HM. The immunological and histopathological changes of tramadol, tramadol/acetaminophen and acetaminophen in male albino rats "Comparative study". Egypt J Hosp Med 2011;45:477-503.*
22. *Hafez EM, Issa SY, Abdel Rahman SM. Parenchymatous toxicity of tramadol: histopathological and biochemical study. Drug Alcohol Depend 2015;3(5):1-6.*
23. *Haschek WM, Rousseaux CG, Wallig MA. Fundamentals of toxicologic pathology, 2nd ed. Academic Press, San Diego; 2010. p. 197-235.*
24. *Neyrinck A. Modulation of Kupffer cell activity: physio-pathological consequences on hepatic metabolism. Bull Mem Acad R Med Belg 2004;159(5-6):336-58.*
25. *Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82(1):47-95.*
26. *Ahmed RM. The histological changes induced by cisplatineum on the liver tissue in rats: The role*

of vitamin C. M.Sc Thesis. Collage of medicine, University of Mosul. 2012;28-29.

27. *Abdulqader SZ, Mustafa IA. The protective role of vitamin C against formaldehyde induced-hepatotoxicity and nephrotoxicity in male rats. J Pharm Biol Sci 2014;9(4):21-6.*
28. *Kini RD, Tripathi Y, Raghuveer CV, et al. Role of vitamin C as an antioxidant in cadmium chloride induced testicular damage. Int J Appl Biol Pharm 2011;2(3):484-8.*